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Nitrogen Fertility and Planting Date Effects on Lint Yield and Cry1Ac (Bt) Endotoxin Production

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ABSTRACT

Early planted cotton (*Gossypium hirsutum* L.) and varieties expressing the *Bacillus thuringiensis* (Bt) gene offer improved yield potential. It is not clear whether the current N recommendations remain appropriate for these new production options. The objectives were to determine how varying rates, application timing, and sources of N affected cotton dry matter partitioning, leaf chlorophyll (Chl) concentration, leaf Bt (Cry1Ac) endotoxin concentration, lint yield, and fiber quality. Four N treatments (112 kg N ha^{-1} anhydrous; 112 kg N ha^{-1} liquid; 112/56 kg N ha^{-1} split; 56/56 kg N ha^{-1} split) were applied to three cotton varieties (PM 1218BR, STV 4691B, and FM 832) planted on either an early or normal planting date from 2001 through 2004. The N response was consistent across planting dates and varieties for all data collected as shown by the lack of any interactions with these variables. Although N treatments had no effect on lint yield or any dry matter partitioning components, plants receiving the 112/56 kg N ha^{-1} split application treatment exhibited 14% greater leaf Cry1Ac concentration and a 3% greater leaf Chl concentration than the other N treatments. Early planted cotton had 5% greater leaf Chl concentration but a 12% lower leaf Cry1Ac concentration than normal planted cotton. Lower Cry1Ac levels may reflect enhanced remobilization of the leaf protein to feed the faster developing boll load of the early planted cotton. Early planted cotton yielded 10% more than normal planted cotton because it produced 9% more bolls. Current N recommendations for normal planted cotton also appear sufficient for early planted cotton.

NITROGEN is widely considered one of the major essential nutrients for plant growth. However, proper N fertilization in upland cotton can often be viewed as more of an art form rather than a science. Application rates decisions often must factor in such variables as soil texture and realistic yield goals (McCarthy and Funderburk, 1990). The N requirement uncertainty for optimal cotton yields under different environmental conditions is due to the indeterminate growth habit of cotton and the complexity of N cycling in the soil (Gerik et al., 1998). While rates above 112 kg N ha^{-1} rarely elicit a positive yield response in the mid-southern U.S. cotton production belt (McConnell et al., 1993), rates as high as 224 kg N ha^{-1} can be recommended in California in fields with lower soil NO_3^- levels but high yield goals (Weir et al., 1996).

Recent research has indicated that planting the cotton crop earlier than has historically been typical in the Mississippi Delta has the potential to increase lint yield production (Pettigrew, 2002). It is not known whether input usages optimized for traditional planting production systems are still appropriate for the early planting production system. For instance, with additional N, could the early planting system take further advantage of its longer growing season to produce even greater yields via more boll production at the top of the canopy? The premature photosynthetic decline of the cotton canopy due to an apparent remobilization of ribulose 1,5 bisphosphate carboxylase-oxygenase (Rubisco) to support the strong developing reproductive sink demand (Pettigrew et al., 2000) suggests that extra and timely N fertilization could maintain the canopy's photosynthetic capacity longer and potentially support even greater yield increases. However, application of this theory also runs the risk of promoting vegetative regrowth, complicating crop management, influencing yield development, and slowing the defoliation process.

An alteration or disruption in the N level could potentially impact protein synthesis and metabolism because N is, among other things, a linkage component in the peptide bonds binding amino acids together into proteins. This issue becomes particularly important in transgenic crops where a technology fee is charged to grow plants containing a gene that expresses a particular transgenic trait. Anything that alters protein synthesis or metabolism could potentially alter expression of a transgenic trait. Bruns and Abel (2003) demonstrated in greenhouse grown maize (*Zea mays* L.) plants that increasing levels of N fertilization resulted in increasing quantities of Bt endotoxin (Cry1Ab) produced in the leaf tissue. The Bt endotoxin is lethal to certain lepidopteran insects. Some of the current transgenic traits available to the U.S. cotton producers include plants containing different genes that produce the Cry1Ac endotoxin, plants with a gene conveying resistance to the herbicide glyphosate, plants with a gene conveying resistance to the herbicide glufosinate, and plants with a gene conveying resistance to the herbicide bromoxynil. To date little research has been performed to investigate how various production practices might affect the level of transgenic trait expression in cotton.

The relative untested effects of the cotton early planting production system dictates that further fine tuning may be necessary to optimize production inputs

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Abbreviations: Chl, chlorophyll; LAI, leaf area index; NAWB, nodes above white bloom; PPFD, photosynthetic photon flux density; SLW specific leaf weight.

inherent with the system. One of the objectives of this study was to determine how varying N amounts, application timing, and sources affected cotton production for multiple cotton varieties when grown under both the conditions of the early planting production system and under a later more traditional planting date. The second objective was to determine how varying planting dates and N fertility regimes affected the expression level of the Cry1Ac endotoxin.

MATERIALS AND METHODS

Field studies were conducted from 2001 through 2004 near Stoneville, MS on a Dubbs silt loam soil (fine-silty, mixed, thermic Typic Hapludalfs). Three different cotton varieties were grown each year of the study. In 2001 through 2002, 'PM 1218BR', 'STV 4691B', and 'FM 832' were grown. Both PM 1218BR and STV 4691B are Bt cotton varieties containing a gene expressing the Cry1Ac endotoxin, with the PM 1218BR also containing a gene providing glyphosate resistance. In 2003 and 2004, 'STV 4892BR' was substituted for STV 4691B. Both STV 4691B and STV 4892BR have 'STV 474' as their recurrent parent with STV 4892BR possessing the glyphosate resistance gene in addition to the Bt gene. In 2004, 'FM 800BR' was substituted for FM 832. FM 832 serves as the recurrent parent for FM 800BR with FM 800BR containing additional genes for both Bt endotoxin expression and glyphosate resistance. The four N treatments consisted of 112 kg N ha^{-1} applied preplant as anhydrous ammonia (112 anhydrous), 112 kg N ha^{-1} applied preplant as a urea-ammonium nitrate solution (112 liquid), 112 kg N ha^{-1} applied preplant plus 56 kg N ha^{-1} applied sidedress as the urea-ammonium nitrate solution (112/56 split), and 56 kg N ha^{-1} applied preplant plus 56 kg N ha^{-1} applied sidedress as the urea-ammonium nitrate solution (56/56 split). The sidedress N applications were made in early to mid-June of each year. Two planting dates were used in this study, early April (Early) and early May (Normal). Early planting of the plots occurred on 2 April in 2001, 4 April in 2002, 31 March in 2003, and 31 March in 2004. Normal planting occurred on 1 May in 2001 and 2002, 30 April in 2003, and 3 May in 2004.

The experimental design used was a randomized complete block with a split-plot treatment arrangement and six replications. The two planting dates were the main plots. The N treatments and varieties were arranged factorially to form the subplots. To minimize N treatment carryover effects from 1 yr to the next, main plots and subplots were randomly assigned in 2001 and then remained in the same location thereafter, with the exception of the variety substitutions previously mentioned. Plots, consisting of four rows spaced 1-m apart and 18.3-m long were planted with the seeding rate of each seed lot adjusted (assuming a 75% germination, emergence, and survival rate) to result in a final plant population density of approximately 97 000 plants ha^{-1}.

Dry matter harvests were taken during 11 thru 15 June and 9 thru 12 July in 2001; during 10 thru 12 June and 22 thru 26 July 2002; during 9 thru 11 June and 21 thru 24 July in 2003, and during 7 thru 9 June and 19 thru 23 July in 2004. On each harvest date, the aboveground portions of plants from 0.3 m of row were harvested from one of the outside plot rows and separated into leaves, stems and petioles, squares, and blooms and bolls. Leaves were passed through a LI-3100 leaf area meter (LI-COR, Lincoln, NE) to determine leaf area index (LAI), and main-stem nodes were counted. Samples were dried for at least 48 h at 60°C, and dry weights were recorded. Harvest index was calculated as the reproductive dry weight/total dry weight.

The percentage of photosynthetic photon flux density (PPFD) intercepted by the canopies of both experiments was determined with a LI 190SB point quantum sensor (LI-COR, Lincoln, NE) positioned above the canopy and a 1-m-long LI 191SB line quantum sensor placed on the ground perpendicular to and centered on the row. Two measurements were taken per plot with the average of those two measurements used for later statistical analysis. All measurements were taken between 1230 and 1430 h CDT with all above canopy reading $\geq$1700 μmol m^{-2} s^{-1}. These PPFD interception data were collected on 13 June and 9 July 2001; 18 June and 18 July in 2002; 24 June and 14 July in 2003; and 12 July in 2004.

The number of white blooms (blooms at anthesis) per subplot were counted on a weekly basis to document the blooming rate throughout the growing season. These counts were taken on 6.1 m of row from one of the inner subplot rows, were initiated at the first sign of blooming, and were continued until production of blooms had virtually ceased. The number of main-stem nodes above a sympodial branch that had a white bloom at the first branch fruiting position (NAWB) were also counted weekly on three plants per plot to document the progressive reproductive development up the stem as well as crop maturity. Bloom counts and NAWB data were collected every year of the study.

Leaf Cry1Ac endotoxin concentration was determined in 2003 on the varieties PM 1218BR and STV 4691B and in 2004 on the varities PM 1218BR and STV 4892BR. Leaf samples from terminal leaves were collected from five healthy plants for all subplots on 8, 16, and 30 July in 2003, and on 19 and 27 July in 2004. Terminal leaves from the plants were used because this tissue accurately reflects overall expression differences among cultivars (Adamczyk and Sumerford, 2001). Tissue was excised from the lobed region of the terminal leaf by placing the tissue underneath the attached cap of a 0.5-mL microcentrifuge tube. Closing the cap produced a uniform circular sample of approximately 4.8 mg that was self-contained within the microcentrifuge tube, while minimizing sample desiccation. The five individual leaf samples per plot were placed into a plastic bag and transported to the laboratory in a cooler with ice. Within 1 h, the five samples per plot were combined into an individual 2.0-mL 96 deep-well microtiter plate (BioSpec Products, Inc., Bartlesville, OK) containing two 6.4-mm stainless steel ball-bearings. Quantification of the levels of Cry1Ac present in the combined samples were made using a commercially available kit (Envirologix, Inc., Portland, ME) by initially adding 1.0-mL Cry1Ac extraction buffer to each well and then homogenizing the tissue samples for 30 s using a Mini-Beadbeater-96 (BioSpec Products, Inc.). The microtiter plate was then centrifuged at 5000 × *g* for 5 min at 4°C (Avanti J-20XP, Beckman Coulter, Inc., Fullerton, CA). For each sample, a 20-μL aliquot was placed in an individual 1.1-mL 96 deep-well microtiter plate containing 500 μL of Cry1Ac extraction buffer (EnviroLogix, Inc.) (1:26 dilution). The microtiter plate was covered with a corresponding silicone-based lid (BioSpec Products, Inc.) and shaken on an orbital shaker for 1 min. A commercial quantification plate kit then was used to quantify the amount of Cry1Ac present for each variety per plot (EnviroLogix, Inc.). Samples were plotted against a standard curve with Cry1Ab calibrators supplied in the kit. A simple conversion was used to express values as "Cry1Ac" as dictated by the kit protocol. The amount of Cry1Ac was expressed as mg kg^{-1} after accounting for the proper dilution factors. Mean expression of the leaf Cry1Ac concentration for each subplot was generated by averaging across the multiple sample dates per year with that average subplot value being used in later statistical analyses.

Table 1. Monthly weather summary for 2001 through 2004 at Stoneville, MS.†

Month	2001	2002	2003	2004
		Precipitation, cm		
April	10.1	8.3	9.6	10.5
May	12.9	7.2	6.5	18.4
June	7.0	10.5	18.5	31.6
July	8.0	8.4	6.2	7.8
August	21.5	7.0	3.9	5.5
September	7.7	19.6	12.5	0.1
October	10.0	17.9	10.1	18.1
		Thermal units‡		
April	145	135	114	107
May	251	214	245	249
June	310	319	288	317
July	395	397	375	362
August	366	378	392	315
September	235	309	248	275
October	77	116	127	203
		Solar radiation, MJ m^{-2}		
April	420	437	474	671
May	559	506	482	663
June	549	523	656	644
July	546	581	692	672
August	462	522	641	657
September	399	378	598	571
October	381	253	476	380

† All observations made by NOAA, Mid-South Agric. Weather Service, and Delta Research and Extension Center Weather, Stoneville, MS.
‡ [(Max. temp + Min. temp.)/2] − 15.5°C.

Leaf chlorophyll (Chl) concentration was determined on the youngest fully mature leaf, usually the fourth or fifth leaf down from the plant terminal, during 2003 and 2004. Leaf discs (0.7-cm diam.) were collected from four leaves per subplot during 24 through 30 July in 2003 and during 23 through 28 July in 2004. The four leaf discs (one per leaf) were placed in 10 mL of 950 mL L^{-1} ethanol and the Chl was extracted over a 24-h period of darkness at 30°C. This extracted Chl was then quantified spectrophotometrically according to the methods of Holden (1976).

Cotton was defoliated using a mixture of tribufos and ethephon during early-to-mid September each year. Defoliation was initiated for all plots when approximately 65% of the bolls in the normal planted plots had opened. Approximately 2 wk after defoliation, the two center rows of each subplot were mechanically spindle-picked and weighed. A final mechanical harvest of all the subplots occurred approximately 2 wk after the first harvest. After defoliation, but before the first mechanical harvest, a 50-boll sample was collected from each subplot for use in determination of yield components. Boll mass was determined from these 50 boll samples by dividing the weight of seed cotton by the number of bolls harvested. These samples were then ginned and weighed to calculate lint percentage which was used to calculate lint yield from the mechanically harvested seed cotton. The number of bolls produced per unit ground area was calculated from the boll mass and total subplot seed cotton weights. Average seed mass was determined from 100 nondelinted seeds per sample and reported as weight per individual seed. Lint samples from each subplot were sent to Starlab Inc. (Knoxville, TN) for fiber quality determinations. Fiber strength (T1) was determined with a stelometer. Span lengths were measured with a digital fibrograph. Fiber maturity, wall thickness, and perimeter were calculated from arealometer measurements.

Statistical analyses were performed by analysis of variance (PROC MIXED; SAS Institute, 1996). Because the planting dates, N fertility treatments and varieties were returned to the same field position each year, year was considered a repeated measure sub-subunit in the analysis. Variety means, N fertility means, and planting date means were averaged across years and each other when statistically important interactions were not detected. Variety, N fertility, and planting date means were separated by use of a protected LSD at $P \leq 0.05$.

RESULTS AND DISCUSSION

Varying weather conditions across the 4 yr of this study provided distinct growing environments each year (Table 1). Excessive rainfall occurred during late August and early September in 2001 that impacted harvest; some lint was blown on the ground and some of the seed germinated in the boll. Milder temperatures in 2003 and 2004, combined with ample rainfall in June to carry the crop through blooming and boll set, and with favorable conditions during harvest produced superior yields in these 2 yr (data not shown).

Similar to previous research (Pettigrew, 2002), the early planted cotton had 136% greater LAI and 68% taller plants than the normal planted cotton in June (Table 2). The canopy of the early planted cotton also intercepted 48% more PPFD at this time than the normal planted cotton. This result was to be expected since the early planted cotton was older and further along in development than the normal planted cotton. At this sampling time, varying the N source, amount or timing of application did not affect any of the dry matter partitioning traits that were monitored. However, the

Table 2. Cotton dry matter partitioning and canopy photosynthetic photon flux densities (PPFD) interception during mid-June as affected by varying planting dates and N fertility treatments averaged across varieties and the years 2001 through 2004. Planting date means are averaged across N treatments and N treatment means are averaged across planting dates.

Planting date	N treatment	Height	Main stem nodes	Height/node ratio	Leaf area index	Specific leaf wt.	Total wt.	Harvest Index†	PPFD interception
	kg ha^{-1}	cm	nodes $plant^{-1}$	cm $node^{-1}$		g m^{-2}			%
Early		32	12.8	2.47	0.71	65.8	50.4	0.033	44.7
Normal		19	8.2	2.36	0.30	62.8	18.1	0.006	30.3
LSD (0.05)		2	0.3	ns‡	0.07	2.7	5.5	0.006	2.7
$P > F$		0.01	0.01	0.24	0.01	0.03	0.01	0.01	0.01
	112 anhydrous	26	10.6	2.41	0.52	63.5	35.0	0.019	37.6
	112 liquid	26	10.4	2.45	0.49	64.7	33.7	0.019	38.9
	112/56 split	25	10.4	2.38	0.50	65.4	32.5	0.017	36.5
	56/56 split	26	10.6	2.41	0.52	63.6	35.7	0.024	36.9
	LSD (0.05)	ns	ns	ns	ns	ns	ns	ns	1.5
	$P > F$	0.73	0.13	0.32	0.71	0.12	0.26	0.47	0.01

† Harvest Index = Reproductive dry weight/total dry weight.
‡ Not significantly different at the 0.05 level.

Table 3. Cotton dry matter partitioning and canopy photosynthetic photon flux densities (PPFD) interception during late July as affected by varying planting dates and N fertility treatments averaged across varieties and the years 2001 through 2004. Planting date means are averaged across N treatments and N treatment means are averaged across planting dates.

Planting date	N treatment	Height	Main stem nodes	Height/node ratio	Leaf area index	Specific leaf wt.	Total wt.	Harvest Index†	PPFD interception
	kg ha^{-1}	cm	nodes plant^{-1}	cm node^{-1}		g m^{-2}			%
Early		93	21.7	4.30	3.42	54.1	566.7	0.262	76.8
Normal		90	18.9	4.69	3.00	51.8	386.7	0.108	72.4
LSD (0.05)		ns‡	0.3	0.23	0.21	ns	54.7	0.027	2.9
$P > F$		0.08	0.01	0.01	0.01	0.06	0.01	0.01	0.01
	112 anhydrous	92	20.3	4.52	3.17	52.5	476.1	0.184	74.6
	112 liquid	92	20.3	4.50	3.29	52.4	477.4	0.182	74.6
	112/56 split	91	20.3	4.49	3.18	53.5	474.4	0.184	75.5
	56/56 split	91	20.3	4.47	3.20	53.4	479.0	0.190	73.8
	LSD (0.05)	ns	ns	ns	ns	ns	ns	ns	ns
	$P > F$	0.92	0.99	0.79	0.86	0.61	0.99	0.86	0.32

† Harvest Index = Reproductive dry weight/total dry weight.
‡ Not significantly different at the 0.05 level.

canopy of the 112 liquid treatment intercepted slightly more PPFD interception than either the 112/56 split or the 56/56 split N fertility treatments.

By late July, vegetative growth of the normal planted plants approached that of the early planted, as there was no difference in plant height between planting dates and the LAI of the early planted cotton was only 14% greater at this time (Table 3). At this time, the early planted cotton had accumulated more of its resources and assimilates in reproductive growth than the normal planted cotton as was demonstrated by the 143% greater harvest index of the early planted cotton compared to the normal planted. Indicative of the narrowing of the LAI difference between planting dates observed since the June harvest date, the amount of PPFD intercepted by the early planted canopies was only 6% greater than the normal planted canopies at the July harvest date. Similar to the results from the harvests in June, no differences among the N fertility treatments were detected for any of the dry matter partitioning data. In addition, the earlier detected canopy PPFD interception differences among the N fertility treatments was no longer observed in July.

Although no N treatment differences were detected for either vegetative or reproductive growth at either sampling time monitored, differences among the N fertility treatments were detected in the NAWB counts, an indirect measure of the progression of reproductive and vegetative growth (Fig. 1 and 2). The additional N provided with the 112/56 split treatment (168 kg total N ha^{-1}) resulted in greater NAWB determinations compared to the other N fertility treatments (112 kg total N ha^{-1}). The 112/56 split N treatment had a significantly greater NAWB than the 112 anhydrous N treatment on four occasions in 2001, but this difference between the treatments was only significant on the last measurement date in 2002 (Fig. 1). There were no NAWB differences among N treatments observed in 2003. However, toward the end of the 2004 season, the 112/56 split treatment again had the highest NAWB level. Earlier in the 2004, the 112 anhydrous N treatment had higher NAWB counts. Because high NAWB levels is indicative of either greater or sustained vegetative growth relative to the reproductive growth, it appears that the 112/56 N treatment was generally able to sustain a level of vegetative growth longer than the other N treatments, presumably due to the extra N applied.

Differing production practices altered the expression of both the leaf Cry1Ac endotoxin concentration and the leaf Chl concentration at the July sampling time in 2003 and 2004 (Table 4). Although the early planted cotton had a 5% greater leaf Chl concentration than the normal planted cotton, planting the cotton early resulted in a 12% lower leaf Cry1Ac concentration relative to that expressed in leaves from normal planted cotton during this period. Similar results were found with cotton leaves from canopies that had reached or not

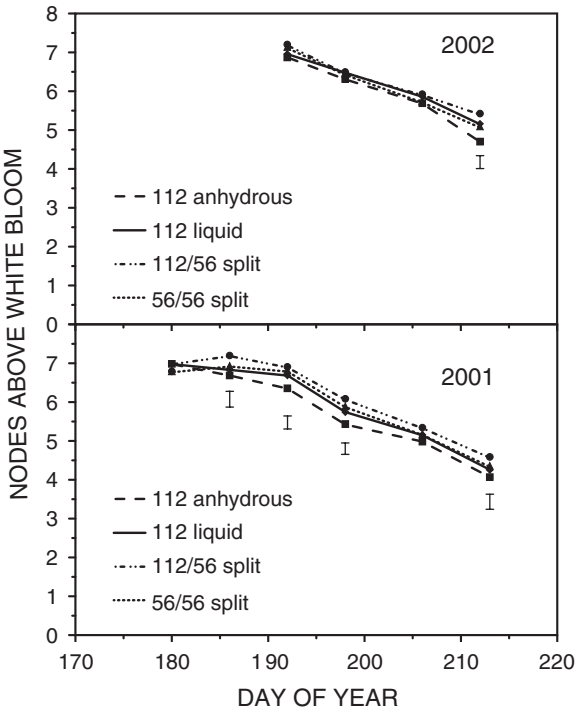

Fig. 1. Number of main-stem nodes of cotton above a sympodial branch with a first-position white bloom (bloom at anthesis) at various times throughout the 2001 and 2002 growing seasons in plots of four different N fertility treatments. These N fertility treatment means were averaged across two planting dates and three cotton varieties. Vertical bars denote LSD values at the 0.05 level and are present only when the differences between N fertility treatments are statistically significant at the 0.05 level.

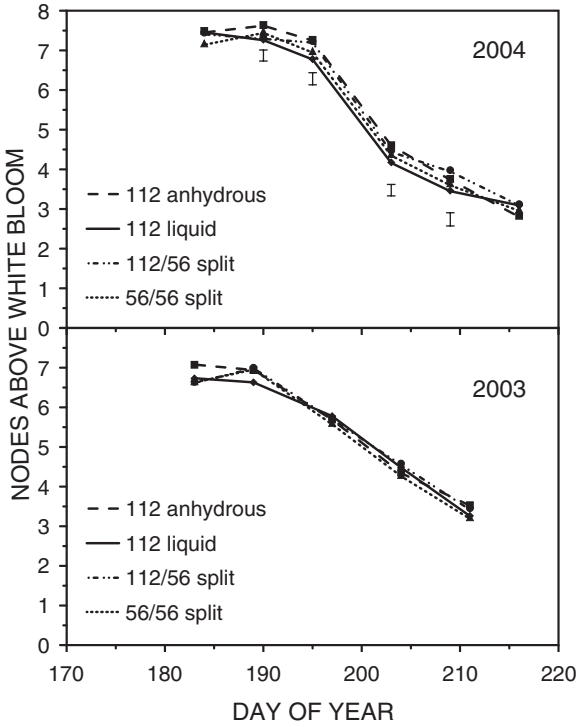

Fig. 2. Number of main-stem nodes of cotton above a sympodial branch with a first-position white bloom (bloom at anthesis) at various times throughout the 2003 and 2004 growing seasons in plots of four different N fertility treatments. These N fertility treatment means were averaged across two planting dates and three cotton varieties. Vertical bars denote LSD values at the 0.05 level and are present only when the differences between N fertility treatments are statistically significant at the 0.05 level.

reached cutout (a slowing of vegetative growth due to increased assimilate demand by the reproductive growth) as no differences were detected in leaf Chl concentrations, but soluble protein concentrations were reduced in leaves from the older canopy leaves (Pettigrew et al., 2000). Although the various N fertility treatments that applied only 112 kg N ha^{-1} total did not differ in either leaf Chl or Cry1Ac concentrations, the 112/56 split treatment that applied 168 kg N ha^{-1} total had the greatest leaf Chl and Cry1Ac concentrations of any of the N fertility treatments.

Varieties differed for leaf Cry1Ac concentration and Chl concentration (Table 5). In 2003, STV 4691B had 8% greater leaf Cry1Ac concentration than PM 1218BR. However, there were no Cry1Ac concentration variety differences detected in 2004 when STV 4892BR was substituted for STV 4691B. Adamczyk and Sumerford (2001) and Adamczyk and Meredith (2004) also reported differences in the level of Cry1Ac expression depending on the genetic background expressing the trait. Leaf Chl also varied among varieties, with the variety differences changing between 2003 and 2004 as the varieties used changed each year. STV 4691B had a 4% greater leaf Chl concentration than PM 1218BR, but the Chl concentration of FM 832 was 5% lower than that of PM 1218BR in 2003. In 2004, STV 4892BR, a sister line of STV 4691B that were both derived from STV 474, similarly exhibited a 6% greater leaf Chl than PM 1218BR. However, in contrast with 2003, FM 800BR, the transgenic version of the recurrent parent line FM 832, had 10% greater leaf Chl concentration than PM 1218BR. Both FM 832 and FM 800BR express the okra leaf-type trait which has previously been associated with greater leaf Chl concentration than their normal leaf-type isogenic pair (Pettigrew et al., 1993; and Pettigrew, 2004).

Lint yields and components of yield were affected by both planting dates and N fertility treatment treatments (Table 6). Similar to previous research (Pettigrew, 2002), the early planted cotton yielded 10% more lint than that produced by the normal planted cotton. The yield component primarily responsible for this early planting yield increase was a 9% increase in the number of bolls produced per unit ground area relative to that of the normal planted cotton. None of the other yield components differed significantly between planting dates at the $P \leq 0.05$ level. No lint yield differences were detected among the N fertility treatments, but some of the yield components were affected by these treatments. Although the 112 anhydrous N treatment produced 5% more bolls per unit ground area than the 112/56 split N treatment, it also adjusted its reproductive sink by producing a smaller boll mass than the other N fertility treatments.

Table 4. Cotton leaf Cry1Ac (Bt) endotoxin concentration, chlorophyll concentration, and chlorophyll A/B ratio as affected by varying planting dates and N fertility treatments, averaged across varieties and the years 2003 through 2004. Planting date means are averaged across N treatments and N treatment means are averaged across planting dates.

Planting date	N treatment	Leaf Cry1Ac (Bt) endotoxin conc.	Leaf chlorophyll conc.	Leaf chlorophyl A/B ratio
	kg ha^{-1}	mg kg^{-1}	g m^{-2}	
Early		2.69	449	3.65
Normal		3.07	429	3.61
LSD (0.05)		0.20	13	ns†
P > *F*		0.01	0.01	0.15
	112 anhydrous	2.72	431	3.63
	112 liquid	2.74	439	3.60
	112/56 split	3.18	448	3.65
	56/56 split	2.89	437	3.63
	LSD (0.05)	0.28	12	ns
	P > *F*	0.01	0.05	0.71

† Not significantly different at the 0.05 level.

Table 5. Cotton leaf Cry1Ac (Bt) endotoxin concentration, chlorophyll concentration, and chlorophyll A/B ratio for various cotton varieties during the years 2003 through 2004. Variety means were averaged across N treatments and planting dates.

Year	Varieties	Leaf Cry1Ac (Bt) endotoxin conc.	Leaf chlorophyll conc.	Leaf chlorophyl A/B ratio
		mg kg^{-1}	g m^{-2}	
2003	FM 832	–	429	3.49
	PM 1218BR	3.47	453	3.55
	STV 4691B	3.74	470	3.45
	LSD (0.05)	0.25	16	0.05
	P > *F*	0.03	0.01	0.01
2004	FM 800BR	–	447	3.77
	PM 1218BR	2.05	405	3.77
	STV 4892BR	2.32	430	3.75
	LSD (0.05)	ns†	14	ns
	P > *F*	0.08	0.01	0.95

† Not significantly different at the 0.05 level.

Table 6. Cotton lint yield and yield components as affected by varying planting dates and N fertility treatments averaged across varieties and the years 2001 through 2004. Planting date means were averaged across N treatments and N treatment means are averaged across planting dates.

Planting date	N treatment	Lint yield	% First harvest	Boll no.	Lint percentage	Boll mass	Seed mass	Seed no.	Lint Index
		kg ha^{-1}	%	boll m^{-2}	%	g	mg	seed boll^{-1}	mg seed^{-1}
Early		1270	96.5	63	40.2	5.15	104.2	29.3	70.2
Normal		1158	92.4	58	39.8	5.14	104.8	29.3	69.3
LSD (0.05)		87	0.7	3	ns†	ns	ns	ns	ns
$P > F$		0.02	0.01	0.01	0.06	0.79	0.37	0.93	0.07
	112 anhydrous	1232	94.7	62	40.2	5.02	102.1	29.0	68.9
	112 liquid	1200	94.2	60	39.9	5.15	104.5	29.4	69.4
	112/56 split	1210	94.2	59	39.8	5.28	106.3	29.7	70.5
	56/56 split	1213	94.6	60	40.0	5.14	105.2	29.0	70.3
	LSD (0.05)	ns	ns	2	ns	0.11	1.5	ns	ns
	$P > F$	0.31	0.54	0.04	0.09	0.01	0.01	0.16	0.07

† Not significantly different at the 0.05 level.

The 112/56 split N treatment, while having fewer bolls m^{-2} than the 112 anhydrous N treatment, produced a larger boll mass than any of the other N fertility treatment. This larger boll mass was due to a greater seed mass than the other N fertility treatments except for the 56/56 split treatment. This shifting of assimilates and resources among the various yield components by the N fertility treatments ultimately cancelled out each other and resulted in no differences in the lint yield.

Altering the planting dates also affected many of the fiber quality traits (Table 7). Fiber elongation was 3% lower in fiber from the early planted cotton compared to the normal planted, which was similar to previously reported results (Pettigrew, 2002). Fiber strength, 2.5% span length, and 50% span length were all reduced 1% in the early planted cotton relative to the normal planted. In addition, micronaire was elevated 4% in the early planted cotton due to a 3% increase in the fiber maturity (a component of micronaire). Other than the fiber elongation differences, the fiber quality differences due to varying planting dates in this study were not detected in the earlier work (Pettigrew, 2002). Even though these fiber quality differences are statistically significant, they are very small biologically and none of the values would fall into the price discount range. Therefore, these differences are most likely of little economic importance.

In general, varying the source, amount, or application timing of the N fertilization did not affect the quality of the fiber produced (Table 7). The lone exception to this generalization is that the fiber strength for the 112 liquid N treatment was significantly greater than the 56/56 split N treatment. None of the other fiber quality traits differed among the N fertility treatments.

The greater lint yield production from the early planted cotton observed in this study reinforces the idea that greater yield potential can be achieved by planting cotton in the Mississippi Delta earlier than has been traditionally been considered normal (Pettigrew, 2002). Presumably, the early planted cotton is able to take advantage of more favorable weather conditions than normal planted cotton. Dong et al. (2004) also reported that shifting the flowering to earlier in the season through transplanting improved yield performance of hybrid cotton grown in China. The fiber quality differences seen with early planted cotton, although all apparently in the wrong direction, would not trigger any price discounts for inferior fiber quality. Therefore, the improved profit potential associated with the greater lint yields of the early planted production system would only be minimally, if any, offset by slightly inferior fiber quality.

Extra N fertilization was not necessary to achieve the early planted yield increases. Furthermore, the extra total N applied with the 112/56 split did not further enhance the improved yield potential of the early planted production system. The slight maturity delay (greater NAWB counts) seen with the 112/56 split N fertility treatment did not decrease the percentage of the lint that was gathered on the first harvest (Table 6). This

Table 7. Cotton fiber quality traits as affected by varying planting dates and N fertility treatments averaged across varieties and the years 2001 through 2004. Planting date means were averaged across N treatments and N treatment means were averaged across planting dates.

Planting date	N treatment	Fiber elongation	Fiber strength	Span length 2.5%	Span length 50%	Length uniformity†	Micronaire	Fiber maturity	Fiber perimeter
	kg ha^{-1}	%	kN m kg^{-1}	cm		%		%	μm
Early		7.3	201	2.91	1.43	49.4	4.78	88.5	48.9
Normal		7.5	203	2.93	1.44	49.3	4.61	86.2	49.2
LSD (0.05)		0.1	1	0.01	0.01	ns‡	0.05	1.2	ns
$P > F$		0.01	0.03	0.01	0.01	0.44	0.01	0.01	0.42
	112 anhydrous	7.3	201	2.91	1.44	49.4	4.68	87.4	48.8
	112 liquid	7.4	204	2.92	1.44	49.4	4.71	87.6	49.0
	112/56 split	7.4	202	2.93	1.44	49.2	4.69	86.9	49.3
	56/56 split	7.4	199	2.92	1.44	49.2	4.71	87.5	49.1
	LSD (0.05)	ns	3	ns	ns	ns	ns	ns	ns
	$P > F$	0.17	0.01	0.10	0.78	0.31	0.73	0.51	0.54

† Length Uniformity = (50% span length/2.5% span length) × 100.
‡ Not significantly different at the 0.05 level.

research further confirms that N fertilization rates above 112 kg N ha^{-1} generally do not produce additional yield increases in the mid-southern USA (McConnell et al., 1993). The extra yield potential provided by the early planted production system was not sufficiently large enough to require additional N fertilization.

This research also documented how different production practices can affect the level of the protein product from a transgenic trait. The reduced Cry1Ac endotoxin concentration in leaves from early planted cotton mirrors the reduced leaf soluble protein concentrations in older cotton canopies compared to younger canopies previously reported (Pettigrew et al., 2000). The lack of planting date differences between leaf Chl concentration even though there were reductions in leaf Cry1Ac concentration (a protein) was also similar to the earlier reported results (Pettigrew et al., 2000). Adamczyk and Sumerford (2001) also reported a decline in leaf Cry1Ac levels as the cotton plants aged. All three studies indicate that remobilization of the leaf protein, including the Bt endotoxin, to feed the developing boll load probably occurred. Because PM 1218BR is a slightly earlier maturity cotton variety than either STV 4691B or STV 4892BR, the lower leaf Cry1Ac levels seen in PM 1218BR relative to either STV 4691B or STV 4892BR may be more reflective of enhanced remobilization of the leaf N for PM 1218BR due to its slightly advanced development rather than a genetic difference in trait expression. The data from this study indicate that extra fertilizer N could temporarily delay or alleviate the remobilization induced decline in leaf Cry1Ac levels, but not increase the lint yield. Although varying production practices altered the leaf Cry1Ac concentration, even the lowest concentration level achieved in this study was still sufficient to be highly effective against the tobacco budworm (*Heliothis virescens* F.), the principle lepidopteran pest targeted by this technology for the Mississippi Delta (J.J. Adamczyk, unpublished data, 2001).

In conclusion, this study reenforces the findings of previous N fertility research across the mid-southern USA that cotton does not benefit from N fertilizer rates above 112 kg N ha^{-1} (McConnell et al., 1993). The early planted crop was not able to take further advantage of its longer growing season and produce an even larger crop with extra N from the 112/56 split treatment. However, the extra N from the 112/56 split did result in an increased Cry1Ac endotoxin concentration relative to the N treatments that only applied 112 kg N ha^{-1}. These results indicate that current N fertility recommendations for cotton planted during a normal planting period also appear to be sufficient for early planted cotton. Environmental factors and management strategies may also need to be reexamined and optimized to ensure the most efficient utilization of future transgenic traits.

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